- *Suggested analytical method (principle and references(s)) for nicotine and the 5 metabolizes in urine;*

Davis and Curvall concluded "Of the two methods (GC/MS and LC/MS) available for the comprehensive determination of nicotine and its metabolites, LC/MS/MS appears to be the preferred method of analysis. Virtually all of the major metabolites of nicotine may be resolved from a single sample injection *via* this method including the direct determination of phase II metabolites. Specificity and sensitivity are effectively addressed using the analytical technique."

Davis, R.A. and Curvall, M., Determination of nicotine and its metabolites in biological fluids: in vivo studies, in Analytical Determination of Nicotine and Related Compounds and their Metabolites, Gorrod, J.W. and Jacob, III, P., Eds., Elsevier, 1999, p 628

Note: The method described above is also referred to as Liquid Chromatography Atmospheric Pressure Ionization Tandem Mass Spectrometry (LC/API/MS-MS). It is perhaps the method most rapidly growing in applications today. It has the capability for high automation and it is estimated that several hundred samples per day could be run if a laboratory were dedicated to that purpose.

- The sample volume required for one determination per subject;

One (1) mL is required for one determination.

Byrd, G. D., K.-M. Chang, et al. (1992). Determination of nicotine and its metabolites in urine by thermospray liquid chromatography/mass spectrometry. The Biology of Nicotine: Current Research Issues. P. M. Lippiello, A. C. Collins, J. A. Gray and J. H. Robinson. New York, Raven Press, Ltd.: 71-83.

In an interlaboratory (Round Robin) study, 15-mL aliquots were supplied to each laboratory. Note: (INBIFO participated in this study.)

Byrd, G.D., Davis, R.A., Vala, E.K., Comparison of methods for determining nicotine and its metabolites in urine, [Poster] Conference on the Society for Research on Nicotine and Tobacco, San Diego, CA, March 24-25, 1995.

- *Expected conc. of nicotine and the 5 metabolites in urine for smokers and nonsmokers with references(s);*

Expected urinary concentration of nicotine and its metabolites in smokers and nonsmokers

	SMOKERS [a]	NON-SMOKERS ‡
Analyte	**Urinary Conc. (ng/mL)** [Mean ± standard deviation of triplicate determinations]	**Urinary Conc. (ng/mL)**
Nicotine†	1220±20	Not normally done because nicotine alone would not be very meaningful because of intrapersonal variance.
trans-3'-hydroxycotinine†	4720±430	
Cotinine†	1450±70	1-30 [b] Note: Cotinine only, does not include glucuronide.
Nicotine-N'-oxide	640±50	
Cotinine-N-oxide	502±27	
Demethylcotinine	228±31	
Nornicotine	60±13	

† These values include the glucuronide conjugates for the respective compounds, which are hydrolyzed prior to the analysis.

‡ There have been few studies of the individual metabolites on non-smokers. This is a detection issue, and perhaps an issue of interest.

(a) Davis, R.A. and Curvall, M., Determination of nicotine and its metabolites in biological fluids: in vivo studies, in Analytical Determination of Nicotine and Related Compounds and their Metabolites, Gorrod, J.W. and Jacob, III, P., Eds., Elsevier, 1999, Table 3, p 628, citing Byrd, G.D., LC-MS/MS method for profiling nicotine and its metabolites in biological fluids [Poster] 44th American Society for Mass Spectrometry and Allied Topics, Portland, OR, May 12-16, 1996.

(b) Benowitz, N. L. (1996) "Cotinine as a biomarker for environmental tobacco smoke." Epidemiol Rev 18(2): 188-204.

The biological half-life for those with references;

Analyte	Half-Life ‡	Reference
Nicotine†	~2 hours.	
***trans*-3'-hydroxycotinine†**	**5.9+/- hours**	**Scherer, G., Jarczyk, L., Heller, W.D., Biber, GA., Neurath, G.B. and Adlkofer, F., Pharmacokinetics of nicotine, cotinine and 3'-hydroxycotinine in cigarette smokers, Klin. Wochenschr (1988), 66 Suppl 11:5-11.**
	12.6 hours	**Curvall et al. (1989) German Research Council on Smoking and Health, 6th Scientific Symposium, Titisee, Germany, Gef. 26-28.** **(I am trying to get a copy of these publications. I should have them nextt week. DEL)**
***trans*-3'-hydroxycotinine-glucuronide**	**18.4+/-1.6**	**Kyerematen, et al., Clin. Pharmacol ther, (1990), 48:641-651.**
	15.7+/-1.0	**Scherer, G., Jarczyk, L., Heller, W.D., Biber, GA., Neurath, G.B. and Adlkofer, F., Pharmacokinetics of nicotine, cotinine and 3'-hydroxycotinine in cigarette smokers, Klin. Wochenschr (1988), 66 Suppl 11:5-11.**
Cotinine†	10-17 hrs.	Kyerematen, G.A. and Vesel, E.S., Metabolism of nicotine, Drug. Metab. Rev., (1991), 23, 3-41.
Nicotine-N'-oxide		
Cotinine-N-oxide		
Demethylcotinine		
Nornicotine		

‡ The value given is the elimination half-life. There are other half-life values such as distribution half-life and elimination half-life. Few of these have been studied significantly other than nicotine itself.

† The half-life is for the free metabolite; methods that determine the separate half-life of the free and glucuronide conjugates are not known. A literature search for this should be conducted.

- *Relevant non-cigarette sources and other confounders for nicotine and its metabolites in urine*

Dietary nicotine is not a significant source of nicotine metabolites for smokers or those exposed to average amounts of ETS.

Siegmund, B., E. Leitner, et al. (1999). "Development of a simple sample preparation technique for the gas chromatographic-mass spectrometric determination of nicotine in edible nightshades (*Solanaceae*)." J Chromatogr A 840: 249-260.

Siegmund, B., E. Leitner, et al. (1999). "Determination of the nicotine content of various edible nightshades (*Solanaceae*) and their products and estimation of the associated dietary nicotine intake." J Agric Food Chem 47: 3113-3120.

Possible confounders are:

1. Smoking non-cigarette products such as cigars and pipes
2. Use of other tobacco products such as chewing tobacco or snuff
3. Use of nicotine-containing smoking cessation devices such as inhalers, patches or gum, or possibly working in an environment where these products are manufactured.

Note: Jacob has recently published a method for the determination of anabasine to be used in studies in association with nicotine metabolites. Anabasine, a minor tobacco alkaloid, will be detected only if a tobacco product is used. This could be a precaution against the use of non-tobacco products influencing the results.